Data Evaluation Record on the aerobic elimination/biodegradation of cetyl pyridinium chloride monohydrate in a simulated activated sludge unit

PMRA Submission Number {.....} EPA MRID Number 48012104

Data Requirement:	PMRA Data Code:	
	EPA DP Barcode:	376391
	OECD Data Point:	OECD 303 A
	EPA Guideline:	835.3240

Test material:

Common name:	Cetyl pyridinium chloride.
Chemical name:	
IUPAC name:	1-Hexadecylpyridinium chloride.
CAS name:	Not reported.
CAS No:	6004-24-6 for monohydrate (123-03-5 for anhydrous).
Synonyms:	Cetylpyridinium chloride.
SMILES string:	

Primary Reviewer: James Breithaupt, Agronomist
Signature:
Antimicrobial Division (AD)
Date: 6/30/10

Risk Assessment and Science Support Branch (RASSB)
James Breithaupt 6/30/10

Final Reviewer: Nader ElkassabanyChief
Signature:
Antimicrobial Division (AD)
Date: 6/30/10

Risk Assessment and Science Support Branch (RASSB)

CITATION: Desmares-Koopmans, M. J. E. 2008. Removal of cetylpyridinium chloride (monohydrate) from water in a simulated activated sludge unit. Unpublished study performed by NOTOX B.V., 's-Hertogenbosch, The Netherlands; sponsored and submitted by Vertellus Health and Specialty Products LLC, Zeeland, Michigan (p. 1). NOTOX Project No.: 488591. Experimental start date June 11, 2008 (initiation of unit operation), and completion date June 26, 2008 (p. 6). Final report issued September 22, 2008.

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EXECUTIVE SUMMARY

This study is classified as unacceptable and does not satisfy the OECD 303A test guideline. The primary reason for rejection is the significant sorption to the glassware that exceeded that of the sewage sludge. This phenomenon should be expected given the chemistry of the compound but some steps may be available to avoid the problem. Also, the pH range was greater than the expected 7.5 ±0.5 units. In addition, no easily-degradable reference compound was used in the study, such as adipic acid, 2-phenyl-phenol, 1-napthol, diphenic acid or 1-naphthoic acid. The results were as follows:

Percent removal of cetyl pyridinium chloride monohydrate (2 mg a.i./L) in activated sludge.

Initial (influent):	67-94%.
Additional after 1 day of exposure (effluent):	≥79% to ≥96%.
Additional after exposure as compared to control:	at least 20%.

Percent removal of cetyl pyridinium chloride monohydrate (2 mg a.i./L) without sludge.

Initial (influent):	71-93%.
Additional after 1 day of exposure (effluent):	76-85%.

This study was conducted to determine the removal of cetyl pyridinium chloride monohydrate in a simulated activated sludge unit over a period of 7 days. The study was conducted according to OECD 303 A, Simulation Test - Aerobic Sewage Treatment: Activated Sludge Units (2001), and in compliance with OECD GLP (1997). The study was conducted at 20.6-22.1°C using all glass Husmann type units under continuous operation. System unit parameters were as follows: storage vessel (influent ≥15 L), dosing pump (0.5 L/hour), aeration chamber (3 L), settling vessel, aeration device/air lift pump, collection vessel (effluent ≥15 L) and aerator.

The inoculum was activated sludge obtained from a municipal sewage treatment plant located at s-Hertogenbosch, The Netherlands. The sludge was pre-conditioned by coarse sieving and adjustment of the suspended solids concentration to 2.5 g/L.

Two system units were utilized. The system unit containing sludge was operated at a sludge retention time of 20 days following introduction of the test compound. A second unit containing no sludge served as a control. The systems were allowed to operate for 6 days prior to treatment; thereafter, both systems were fed daily, via influent, with cetyl pyridinium chloride monohydrate at 2 mg a.i./L for six days. Single samples (2 mL, 0.2-µm filtered) of influent were collected at 0, 1, 2, 3, 4, 5 and 6 days posttreament, with effluent samples taken the day following collection of the influent samples. Samples were analyzed for cetyl pyridinium chloride monohydrate by reverse-phase HPLC; the limit of detection was 0.0254 mg/L.

For the unit containing sludge following treatment, suspended solids and dissolved oxygen were 1.5-2.5 g/L and 7.7-8.4 mg/L, respectively; while the pH was measured at 5.6-6.1, then adjusted

to 7.5-7.7. For the control unit (no sludge) following treatment, dissolved oxygen and pH were 8.0-8.3 mg/L and 7.8-8.0, respectively.

In both the unit containing sludge and the control (no sludge), cetyl pyridinium chloride monohydrate was only detected at 0.120-0.660 mg/L (6-33% of nominal applied) in influent samples. In effluent samples, collected 1 day after influent samples, the parent compound was detected at 0.0600-0.124 mg/L (3-6% of nominal) in the control unit, but not detected (<0.0254 mg/L) in the unit containing sludge. Based on initial measured levels of the parent compound in the influents, cetyl pyridinium chloride monohydrate removal following exposure was determined to be 76-85% in the control unit and ≥79% to ≥95% in the unit containing sludge.

The study author attributed the removal of cetyl pyridinium chloride monohydrate in the influents (both units) and the effluent of the control unit (no sludge) to adsorption to the laboratory equipment, with additional adsorption to sludge when present. Consequently, the sludge resulted in additional removal of at least 20% of the parent compound as compared to the control.

Typical validation criteria were not utilized in the conduct of this study; the control unit contained no sludge and a readily biodegradable (reference) substance was not included.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted in accordance with OECD Guideline for the Testing of Chemicals, Section 3, Degradation and Accumulation, "Simulation Test - Aerobic Sewage Treatment: No. 303 A: Activated Sludge Units" (2001); European Economic Community Directive 88/303/EEC, Official Journal of the European Communities No. L133 (1988); and ISO Standard 11733, Water Quality - Evaluation of the elimination and biodegradability of organic compounds in an aqueous medium - Activated sludge simulation tests (2004; p. 6). The following significant deficiencies were noted:

Significant levels of cetyl pyridinium chloride monohydrate (67-94% of nominal applied) were apparently lost, through adsorption to laboratory equipment, prior to introduction into the operating units, and there were no reported attempts, such as silanization of glassware, to prevent adsorption of the test compound.

The unit with the sludge was not maintained at pH 7.5 ± 0.5 during the study. At 1, 3 and 6 days posttreatment, the pH was measured at 5.6-6.1, then adjusted to 7.5-7.7.

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COMPLIANCE: This study was conducted in compliance with OECD Good Laboratory Practice Guidelines (1997, p. 2B). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-2B, 4).

A. MATERIALS:

1. Test Material: Cetyl pyridinium chloride monohydrate (p. 7).

Chemical Structure:

Description: Technical, solid.

Purity: 100%; however, supporting certificate of analysis was not provided.

Lot/Batch No.: 00227993.

Storage conditions of test chemical: Stored at room temperature in darkness.

Physical-chemical properties of cetyl pyridinium chloride monohydrate:

Parameter	Value	Comment
Molecular formula	$C_{21}H_{38}NCl(H_2O)$	
Molecular weight	358.1 g/mol	
Theoretical Oxygen Demand (ThOD)	Not reported.	
Physical appearance	White to off-white powder.	
Water solubility	32-33%	
Hydrolysis	Reported as stable in water for at least 48 hours.	
Melting point	80°C	
Vapor pressure	Not reported.	
UV Absorption	Not reported.	
Dissociation constant (pKa)	Not reported.	
Partition coefficient (octanol/water) K_{ow}/log K_{ow}	Not reported.	
Stability of compound at room temperature	Stable at room temperature in darkness.	

Data obtained from p. 7 of the study report.

2. Reference Standard: A reference standard was not utilized.

Chemical Structure:

Description:

Purity:

Lot/Batch No.:

3. Inoculum: The inoculum was activated sludge obtained from the Waterschap de Maaskant municipal sewage treatment plant, which treats predominantly domestic sewage, located at 's-

Hertogenbosch, The Netherlands (p. 8). The sludge was described as "freshly obtained". Collection methods and a collection date were not reported. Collected sludge was continuously aerated until use.

4. Mineral medium:

Table 1: Preparation of mineral medium.

Description	Reagent	Reagent concentration (mg/L)	Comment
Adjusted ISO medium	$CaCl_2 \cdot 2H_2O$	211.5	
	$MgSO_4 \cdot 7H_2O$	88.8	
	$NaHCO_3$	46.7	
	KCl	4.2	
Preparation	Medium was prepared using tap water purified by reverse osmosis (GEON Waterbehandeling, Berkel-Enschot, The Netherlands); total volume prepared was not reported.		

Data were obtained from p. 8 of the study report.

5. Synthetic sewage:

Table 2: Preparation of synthetic sewage.

Description	Reagent	Reagent concentration (g/L)	Comment
Synthetic medium	Peptone	16	
	Meat extract	11	
	Urea	3	
	K_2HPO_4	2.8	
	NaCl	0.7	
	$CaCl_2 \cdot 2H_2O$	0.4	
	$MgSO_4 \cdot 7H_2O$	0.2	
Preparation	Medium was prepared using Milli-Q water, with the pH adjusted to 7.5 ± 0.5, as needed.		

Data were obtained from p. 9 of the study report.

B. EXPERIMENTAL CONDITIONS:

1. Preliminary experiments: None reported.

2. Pre-conditioning of inoculum: The sludge was coarsely sieved (mesh size not reported) and washed with ISO medium (method not further described, p. 8). The initial suspended solids concentration was reported as 3.8 g/L (as specified by the municipal sewage plant). The sludge was allowed to settle for 30 minutes, then the suspended solids concentration was adjusted to 2.5 g/L (method was not reported).

3. Experimental conditions:

Table 3: Study design.

Parameter		Details
Duration of the test (posttreatment)		7 days.
Suspended solids		1-3 g/L.
Synthetic sewage:mineral medium ratio		1:100 (v:v).
Application rate (mg a.i./L)		2 mg a.i./L.
Control conditions, if used		Test system treated with cetyl pyridinium chloride monohydrate, but containing no sludge.
No. of replications	Control:	Single system.
	Treated:	Single system.
Test apparatus (type/material/volume)		All glass Husmann type unit consisting of a storage vessel (influent ≥15 L), dosing pump (capacity 0.5 L/hour), aeration chamber (3 L), settling vessel, aeration device/air lift pump, collection vessel (effluent ≥15 L) and aerator (Figure 1, p. 10). Operation of the units was continuous.
Renewal/removal of influent/effluent		Daily.
Cycle		150 mL sludge was removed from the system aeration chamber daily, equivalent to 1/20 part of the sludge present in the unit; sludge retention time (SRT) 20 days.
Pre-treatment		Prior to treatment, the unit containing sludge and the control unit containing no sludge were set up and operated for 6 days to allow acclimation. For unit containing sludge, 375 mL sludge was removed from aeration chamber daily, equivalent to 1/8 part of sludge present; SRT 8 days.
Test solution preparation		Cetyl pyridinium chloride monohydrate stock solutions were prepared at 6 mg a.i./L in mineral medium using ultrasonic dispersion (15-17 minutes) and mixing (10 minutes). 5 L stock solution was then diluted with 10 L mineral medium to yield a nominal concentration of 2 mg a.i./L. Final test solution was clear and colorless. Test solutions were prepared daily.
Test material application method	Volume of the test solution used/treatment:	15 L.
	Application method:	Added daily to test system storage (influent) vessel.
Any indication of the test material adsorbing to the walls of laboratory equipment or the test apparatus?		Yes. Cetyl pyridinium chloride was reported as "known to be stable" yet concentrations in the influents of the units with and without sludge were measured at 0.120-0.660 mg/L (6-33% of nominal) and 0.137-0.586 mg/L (7-29% of nominal), respectively. The study author suspected adsorption to laboratory equipment.
Experimental conditions	Temperature (°C):	Not reported.
	Light/darkness:	Not reported.
Other details, if any		None.

Data were obtained from pp. 5, 8-9; Figure 1, p. 10; Tables 1-2, pp. 12-13; Table 3, p. 15 of the study report.

4. Supplementary experiments: None reported.

5. Sampling:

Table 4: Sampling details.

Criteria		Details
Sampling intervals (posttreament)[1]	Influent:	0, 1, 2, 3, 4, 5 and 6 days.
	Effluent:	1, 2, 3, 4, 5, 6 and 7 days.
Sampling method		A single sample (volume not reported) was taken at each collection interval. Samples were filtered (0.2-µm) and the initial 5 mL discarded, then a 2-mL sample was retained.
Method of collection of CO_2 and organic volatile compounds		Not applicable.
Sampling intervals/times for:		
Suspended solids:		Daily six days prior to treatment, and at 0, 2, 6 and 7 days posttreatment.
Dissolved oxygen and pH:		At 4 and 1 days prior to treatment, and at 1, 3 and 6 days posttreatment.
Sample storage before analysis		Filtered samples were stored frozen (temperature not reported) until analysis.
Other observation, if any		Additional reserve samples (2 mL) were collected for possible analysis, if needed.

1 Effluent samples taken one day after influent samples.
Data obtained from p. 11; Tables 1-2, pp. 12-13; Tables 4-5, pp. 15-16 of the study report.

C. ANALYTICAL METHODS:

Extraction/clean up/concentration methods: Media samples were filtered (0.2-µm, S&S FP 030/0.2 CA-S) upon collection (p. 11). Prior to analysis, the frozen, filtered samples were thawed at room temperature, diluted 1:1 (v:v) with 30mM tetrabutylammonium bromide (TBABr) in acetonitrile, then mixed using ultra-sonication for 5 minutes (p. 21).

Identification and quantification of parent compound: Prepared samples were analyzed by reverse-phase HPLC under the following conditions: Waters µBondapak phenyl column (3.9 x 300 mm, 10 µm), column temperature 60°C, isocratic mobile phase of 30mM TBABr and 25mM potassium dihydrogen phosphate (KH_2PO_4) in Milli-Q water (pH 2.5):acetonitrile (50:50, v:v), injection volume 100 µL, flow rate 0.7 mL/minute, UV detector (258 nm; pp. 21-22; Figures 1-2, p. 27). Procedural recoveries for samples fortified at 0.501 and 2.00 mg/L were 97-98% (n = 2) and 98-99% (n = 2), respectively (Table 1 in Appendix II, p. 25).

Detection limits (LOD, LOQ) for the parent compound: The limit of detection for cetyl pyridinium chloride monohydrate was reported as 0.0254 mg/L (Table 1, p. 12).

II. RESULTS AND DISCUSSION

A. TEST CONDITIONS:

In the unit with sludge following treatment, suspended solids were 1.5-2.5 g/L and dissolved oxygen was 7.7-8.4 mg/L; while the pH was measured at 5.6-6.1 and adjusted to 7.5-7.7 using 1N NaOH (Tables 4-6, pp. 15-16). In the unit without sludge (control) following treatment, dissolved oxygen and pH were 8.0-8.3 mg/L and 7.8-8.0, respectively. Temperature during the study was 20.6-22.1°C (p. 14). For both units, system parameters posttreatment were comparable to values measured prior to treatment (Tables 4-6, pp. 15-16).

B. ANOMALIES:

Possible adsorption of the test compound to the laboratory glassware and equipment; see section **C. SUMMARY** below.

C. SUMMARY:

In the unit with sludge, cetyl pyridinium chloride monohydrate was detected at 0.120-0.660 mg/L (6-33% of the nominal applied) in the influent samples and was not detected (<0.0254 mg/L) in effluent samples taken 1 day posttreatment. Consequently, 67-94% of the initial parent was removed during preparation of the treated influent, with additional removal of ≥79% to ≥95% following exposure (Table 1, p. 12).

In the unit without sludge (control), cetyl pyridinium chloride monohydrate was detected at 0.137-0.586 mg/L (7-29% of the nominal applied) in the influent samples and at 0.0600-0.124 mg/L (3-6% of nominal) in effluent samples taken 1 day later. During preparation of the treated influent, 71-93% of initial cetyl pyridinium chloride monohydrate was removed, with additional removal of 76-85% after exposure; excluding the day 3 result of 12% which was considered an outlier (Table 2, p. 13).

The study author reported cetyl pyridinium chloride monohydrate as "known to be stable (no degradation)" and thus attributed the removal of the parent compound in the influent solutions and the effluent solution with no sludge present to adsorption to the laboratory equipment, with additional adsorption to the sludge when present (p. 5; Tables 1-2, pp. 12-13). The presence of the sludge resulted in an additional removal of at least 20% as compared to the control.

Table 5. Percent removal of cetyl pyridinium chloride monohydrate in simulated activated sludge unit.

Day	Sample type[1]	Unit with Sludge		Unit without Sludge (control)	
		CPC[2] (mg/L)	% Removal[3]	CPC[2] (mg/L)	% Removal[3]
0	Influent	0.200	≥87	0.394	85
	Effluent	<LOD[4]		0.0600	
1	Influent	0.494	≥95	0.528	80
	Effluent	<LOD		0.107	
2	Influent	0.524	≥95	0.458	80
	Effluent	<LOD		0.0900	
3	Influent	0.120	≥79	0.137	12
	Effluent	<LOD		0.121	
4	Influent	0.660	≥96	0.586	79
	Effluent	<LOD		0.124	
5	Influent	0.385	≥93	0.501	76
	Effluent	<LOD		0.119	
6	Influent	0.359	≥93	0.427	79
	Effluent	<LOD		0.0900	

1 Effluent sample taken one day after influent sample.
2 Cetyl pyridinium chloride monohydrate.
3 Calculated by study author as (Conc. influent - Conc. effluent) x Conc. effluent x 100%
4 Limit of detection = 0.0254 mg/L.
Data obtained from Tables 1-2, pp. 12-13 of the study report.

D. SUPPLEMENTARY EXPERIMENT-RESULTS:

None reported.

E. VALIDATION:

Typical validation criteria were not utilized in this study:

- This test (Aerobic Sewage Treatment: Activated Sludge Units) is typically conducted with two units, both containing sludge, with one of the units treated with the test compound. A second unit not treated with the test compound serves as the control to determine biodegradation of the organic medium, with dissolved organic carbon (DOC) or chemical oxygen demand (COD) elimination of >80% after two weeks establishing the test as valid. In this study the control unit contained no sludge and was treated with the test compound.

- If an additional unit had been treated with a readily biodegradable (reference) substance, then biodegradation of that substance of >90% would establish the test as valid.

III. STUDY DEFICIENCIES

The following deficiencies were noted:

- Significant levels of cetyl pyridinium chloride monohydrate (67-94% of nominal applied) were apparently lost, through adsorption to laboratory equipment, prior to introduction into the operating units, and there were no reported attempts, such as silanization of glassware, to prevent adsorption of the test compound.

- The unit with the sludge was not maintained at pH 7.5 ± 0.5 during the study. At 4 and 1 days prior to treatment and at 1, 3 and 6 days posttreatment, pH was measured at 5.4-6.1, then adjusted to 7.4-7.7 using 1N NaOH (Table 5, p. 16).

IV. REVIEWER'S COMMENTS

1. Although not required, use of a reference substance, such as adipic acid, 2-phenyl-phenol, 1-napthol, diphenic acid or 1-naphthoic acid, would have been useful to establish that the experimental procedures used in this study were conducted appropriately.

2. The study author reported cetyl pyridinium chloride monohydrate as "known to be stable (no degradation)"; however, supporting results were not provided.

V. REFERENCES

1. U.S. Environmental Protection Agency. 2008. Fate, Transport and Transformation Test Guidelines, OPPTS 835.3240, Simulation Test-Aerobic Sewage Treatment: A. Activated Sludge Units. Office of Prevention, Pesticides and Toxic Substances, Washington, DC. EPA 712-C-08-004.

2. OECD Guideline for Testing of Chemicals. 2001. Simulation Test - Aerobic Sewage Treatment: Activated Sludge Units. 303 A. Adopted by the Council on 22nd January 2001.